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— SECTION E —

MEDICAL SCIENCES

Contents

Page

The Non-availability of Lysine from the Corresponding Hydantoin in the Rat—*Edouard Page, Roger Gaudry, and R. Gingras* 207

The Specificity of the Precipitin Reaction, as Used in the Study of Mosquito Feeding Habits—*W. A. Riddell, J. G. Rempel, and Elspeth McNelly* - - - - - - - - - - - - - 210

Effect of Dietary Protein on Susceptibility of Rats to Trauma —*C. Gwendoline Toby and R. L. Noble* - - - - - - - 216

Influence of Replacement Therapy on the Increased Susceptibility of Protein-deficient Rats to Trauma—*C. Gwendoline Toby and R. L. Noble* - - - - - - - - - - - - 227

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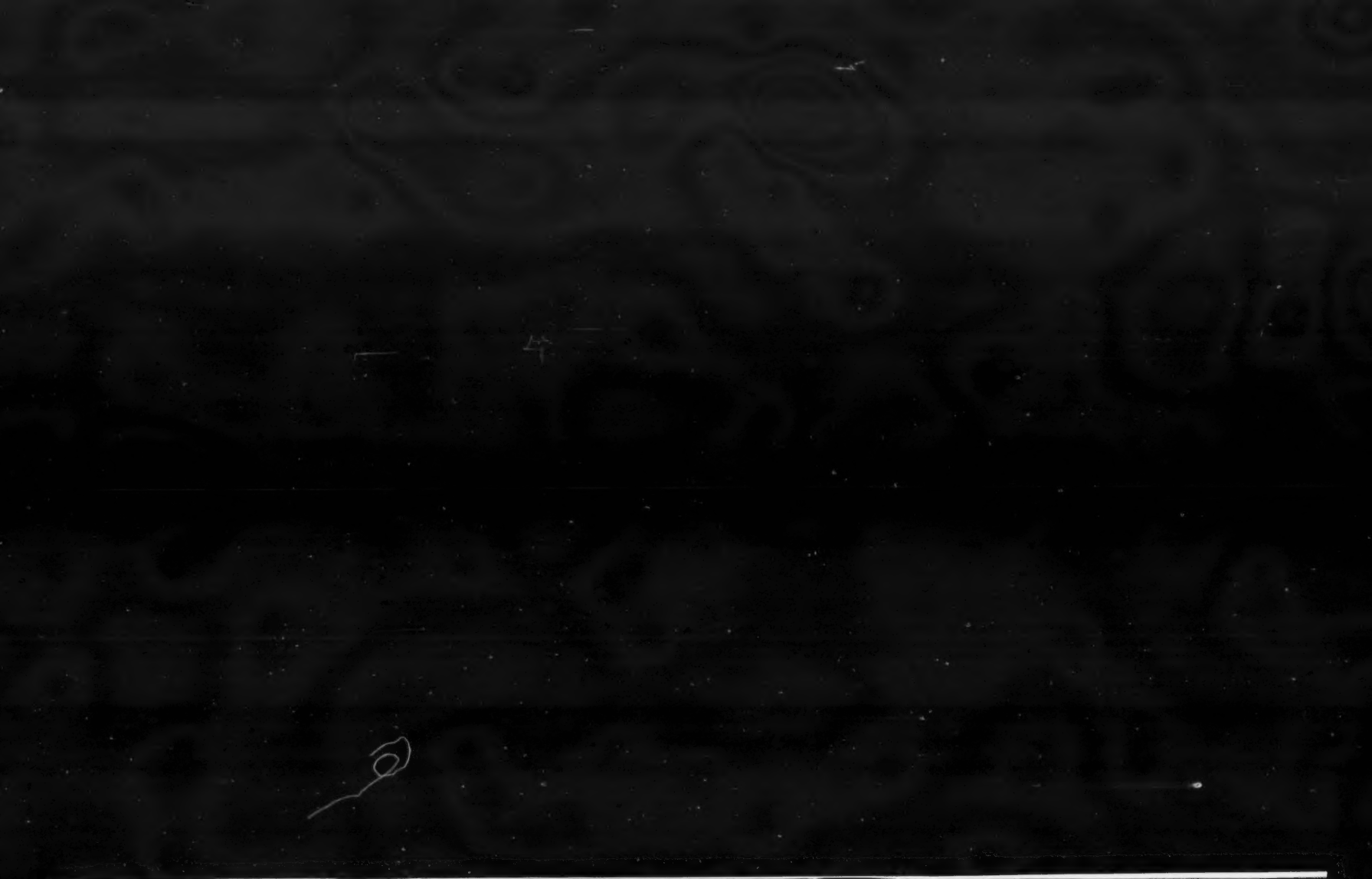

Canadian Journal of Research

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VOL. 25, SEC. E. DECEMBER, 1947 NUMBER 6

THE NON-AVAILABILITY OF LYSINE FROM THE CORRESPONDING HYDANTOIN IN THE RAT

BY EDOUARD PAGE,[2] ROGER GAUDRY,[3] AND R. GINGRAS[4]

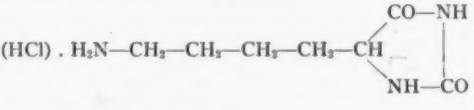
Abstract

The hydantoin of lysine was fed to rats on a lysine-free diet and was shown to have no growth promoting effect. This hydantoin, therefore, does not appear to give rise to the corresponding α-amino acid in contrast with results obtained elsewhere with diphenylhydantoin in man and dog.

Introduction

In 1943, Kozelka and Hine (4) noted that following the intravenous administration of dilantin (diphenylhydantoin) to men and dogs, 1 to 4% of the drug is excreted unchanged, 1 to 5% as the hydantoic acid, and 10 to 27% as α-aminodiphenylacetic acid. Only 30 to 35% of the administered drug could be accounted for. On the basis of these findings and in the absence from the urine of several other possible derivatives, the authors conclude that the mechanism of degradation of diphenylhydantoin is probably as follows: (*a*) a rupture of the ring between the 3 and 4 positions, giving rise to the hydantoic acid, and (*b*) the splitting off of the carbamino group to form the corresponding α-amino acid. Later, Bernheim and Bernheim (2) demonstrated the presence in the tissues of rats and other animals of an enzyme capable of hydrolyzing hydantoin but not diphenylhydantoin. The authors point out that failure to hydrolyze the latter compound may be due to its insolubility in water at physiological hydrogen ion concentration. Bernheim (1) has recently reported that the enzyme in the livers of omnivores that hydrolyzes hydantoin will not hydrolyze any of several substituted hydantoins tested.

In the course of a new method of synthesis of lysine (3), one of us prepared the hydantoin of this amino acid (5-δ-aminobutylhydantoin) and the opportunity was taken of feeding this compound to rats on a lysine-free ration.



[1] *Manuscript received July 15, 1947.*

Contribution from the Department of Biochemistry of the Faculty of Medicine, Laval University, Quebec, Que.

[2] *Assistant.*

[3] *Assistant Professor of Biochemistry.*

[4] *Professor of Biochemistry.*

It was assumed that any substantial conversion of the hydantoin to the corresponding amino acid (lysine) would elicit a marked growth response.

Experimental

Weanling male albino rats were put on a ration of the following composition: zein, 17.4; *dl*-trytophane, 0.6; soybean oil, 4.0; mineral mixture, 4.0; Celluflour, 2.0; Cerelose, 71.85; and choline chloride, 0.15 gm. %*. The food contained the following vitamins: thiamine hydrochloride, 0.4; riboflavin, 0.4; pyridoxine hydrochloride, 0.3; calcium pantothenate, 3.0; nicotinic acid, 3.0; 2-methyl-1,4-naphthoquinone, 0.1; and inositol, 10 mgm. %*. A vitamin A and D concentrate enriched with tocopherol was given by mouth once a week. At the end of 14 days the rats were divided into three groups. Group I (five rats) received the same ration as before; Group II (six rats) received 1.5% *dl*-lysine hydantoin; Group III (six rats) received 1.5% *dl*-lysine. Substitutions were made at the expense of the Cerelose. Fig. 1 shows the growth curves recorded. It is clear that whereas the addition of lysine resulted in an immediate resumption of growth, the hydantoin had no effect either favorable or adverse. The rats receiving this compound behaved exactly like the negative controls.

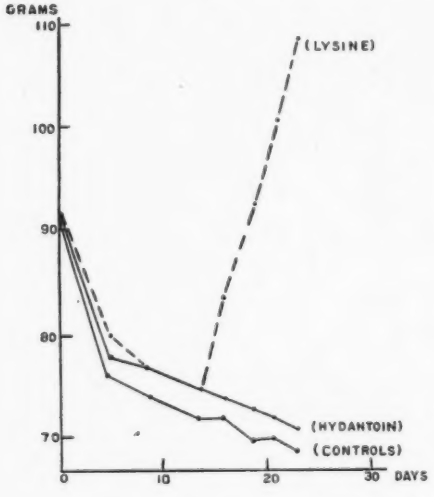


FIG. 1.

Discussion and Conclusion

While these results shed no light on the possible conversion of the hydantoin to hydantoic acid, they do show that if the latter is formed, its further degradation to the corresponding amino acid does not occur to any significant extent,

* *Per 100 gm. of ration.*

if at all. To that extent, these observations are at variance with those of Kozelka and Hine concerning the fate of diphenylhydantoin in man and dog.

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THE SPECIFICITY OF THE PRECIPITIN REACTION, AS USED IN THE STUDY OF MOSQUITO FEEDING HABITS[1]

By W. A. Riddell,[2] J. G. Rempel,[3] and Elspeth McNelly[4]

Abstract

Reared *Aedes aegypti* (Linnaeus) mosquitoes fed under controlled conditions in the laboratory are used to determine the degree of specificity of the precipitin reaction as used in the study of mosquito feeding habits. Three hosts for which antisera have been prepared are used, namely, human, rabbit, and guinea pig. The experiments disclose (1) a high degree of specificity (over 95%) for the precipitin reaction as used in this connection, (2) that multiple reactions signify multiple feedings, and (3) that significant results can be secured by applying the test to undistended mosquitoes from a field collection.

The precipitin reaction has been used extensively in studies connected with the feeding habits of mosquitoes. Nuttall (4) in 1904 established the importance of the reaction in the identification of unknown bloods. Uhlenhuth *et al.* (7) first used it for the identification of the blood meal of insects and suggested its use in the epidemiological studies of insect borne diseases.

During the summer of 1945 a study of the feeding habits of Saskatchewan mosquitoes was made through the application of this test (6). This study disclosed that a remarkably large number of mosquitoes gave a positive reaction to two, or on occasion, even to three antisera. Owing to the fact that none of these multiple reactions was a recognized cross-species reaction, it appeared that among our mosquitoes multiple feeding was common. This emphasized the role mosquitoes might play in the transmission of western equine encephalomyelitis and suggested one explanation of the sporadic epizoötics and epidemics of the disease. Our outbreaks have generally coincided with hot seasons when mosquito activity would be such as to make multiple feeding likely. Some workers (1) have recorded findings that are in agreement with ours, but on the other hand, many researchers record predominantly single reactions. It was then necessary to determine the validity of the multiple readings obtained by the precipitin reaction. Hence, it was decided to carry out extensive tests on known feedings of reared specimens to throw further light on the reliability of the test as applied in this problem.

Materials and Methods

Since our native species of mosquitoes do not feed readily in captivity, especially if unmated, it was decided to use *Aedes aegypti* (Linnaeus), a species

[1] *Manuscript received July 12, 1947.*

This investigation is part of a co-operative survey of encephalomyelitis in Saskatchewan by the Provincial Department of Public Health, the Division of Entomology of the Dominion Department of Agriculture and the Department of Biology of the University of Saskatchewan.

[2] *Director of the Division of Laboratories, Saskatchewan Department of Public Health.*

[3] *Associate Professor of Biology, University of Saskatchewan.*

[4] *Assistant Bacteriologist, Division of Laboratories, Saskatchewan Department of Public Health.*

admirably suited for laboratory experiments. Eggs of this species were secured from Orlando, Fla., through the courtesy of the United States Department of Agriculture, and were shipped to Regina via air mail.

The larvae were reared in aquaria in ordinary lake water at room temperature (75° F.). The pupae were removed to pint jars each covered with a beaker resting on cardboard. A circular opening was made in the cardboard by cutting a series of teeth in the paper and then bending them upwards. It was found that this permitted the adults to enter the beaker readily and also prevented drowning. Adults, both males and females, were transferred to a large resting cage where they were left for 24 hr. These cages were made from ordinary aquaria with the glass ends replaced by masonite. Each end had a circular opening of 5 in. diameter into which was fitted a sleeve of bobbinet. These openings permitted the transfer of flies to the cage and later to individual vials. The top was tightly closed with a board of masonite that slid in a metal channel on each side. At the end of 24 hr. the vials were 'loaded', each with one female specimen. The open end of the vial was covered with bobbinet securely held in place by a rubber band. Rubber gloves were worn during loading to prevent feeding on the laboratory worker (Fig. 1). Each specimen was now assigned a code number and the number entered on the data sheet. Two sets of data sheets were prepared. On one, complete feeding records were entered for each specimen, while the other merely had the specimen code numbers for the technician who read the tests. The technician's findings were later transcribed onto the first set of data sheets. Table I is a sample data sheet with the precipitin test readings entered.

Three animals, for which antisera had been previously prepared, were used in the experiment, namely, man, rabbit, and guinea pig. No serious difficulties were encountered in inducing the mosquitoes to feed. Best results were obtained by tapping the vial gently to cause the mosquitoes to alight on the bobbinet before applying the vial to the experimental animal. In the case of the rabbit all feeding was done on the ear, while with the guinea pig the insects were applied to the back, which had been closely clipped. Human feedings were secured by applying the insect to the forearm.

The following types of feeding were made:

1. Negative: unfed specimens were pressed onto filter paper as described below.

2. Single: on any one of the three hosts.

3. Double: all possible combinations on any two of three hosts, varying the order of feedings and the time interval between the feedings.

4. Triple: on all three hosts, varying the order of feeding and the time interval between the feedings.

The mosquitoes were kept for varied lengths of time after the last feeding. The insects were then etherized, the abdomens severed, and the contents pressed out on a good quality of analytical filter paper, which gave negative

TABLE I

SAMPLE DATA SHEET. LABORATORY FEEDINGS OF REARED *Aedes aegypti* WITH CORRESPONDING PRECIPITIN TEST READINGS, 1946

Specimen code no.	First feeding				Hours between feedings	Condition of abdomen prior to second feeding	Second feeding				Hours between feedings	Third feeding				Hours between last feeding and killed	Killed			Remarks	Precipitin reaction			Remarks
	Date	Time	Host	Extent			Date	Time	Host	Extent		Date	Time	Host	Extent		Date	Time	Condition of abdomen		Incubation, min. 10	20	30	
A349	7-VI	11:00 a.m.	R	w	24	Well distended	8-VI	11:00 a.m.	H	a						1+	8-VI	12:10 p.m.	Partly distended	Second feeding unsuccessful	R			Missed human
A350	7-VI	1:15 p.m.	G	w	22+	Well distended	8-VI	11:30 a.m.	H	g						46	10-VI	9:30 a.m.	Normal		GH			Note human only attempted feeding
A351	7-VI	11:15 a.m.	R	g	24+	Well distended	8-VI	12:00 noon	G	g	23+	9-VI	11:15 a.m.	H	g	22+	10-VI	9:30 a.m.	Partly distended		GH	R		
A352	7-VI	11:15 a.m.	R	g	24+	Well distended	8-VI	11:30 a.m.	H	a						27+	8-VI	12:15 p.m.	Partly distended	Second feeding attempt only	HR			
A353	7-VI	5:30 p.m.	H	g	18+	Very distended	8-VI	11:45 a.m.	R	g						45+	10-VI	9:30 a.m.			HR			
A354	7-VI	11:15 a.m.	R	g	24+	Very distended	8-VI	12:00 noon	G	a	23+	9-VI	11:45 a.m.	H	p	21+	10-VI	9:30 a.m.	Partly distended		HGR			
A355	8-VI	11:45 a.m.	H	g												45+	10-VI	9:30 a.m.	Normal		H			
A356	10-VI	11:00 a.m.	R	g												28	11-VI	3:00 p.m.	With fresh blood		R			
A357	10-VI	11:00 a.m.	R	g	24	Distended	11-VI	11:00 a.m.	H	g						26+	12-VI	1:15 p.m.	Partly distended		HR			
A358	10-VI	11:20 a.m.	R	g												26+	11-VI	3:00 p.m.	With fresh blood		R			

Note: H = human; G = guinea pig; R = rabbit; a = attempt; w = well fed; p = partial; g = gorged.

PLATE I

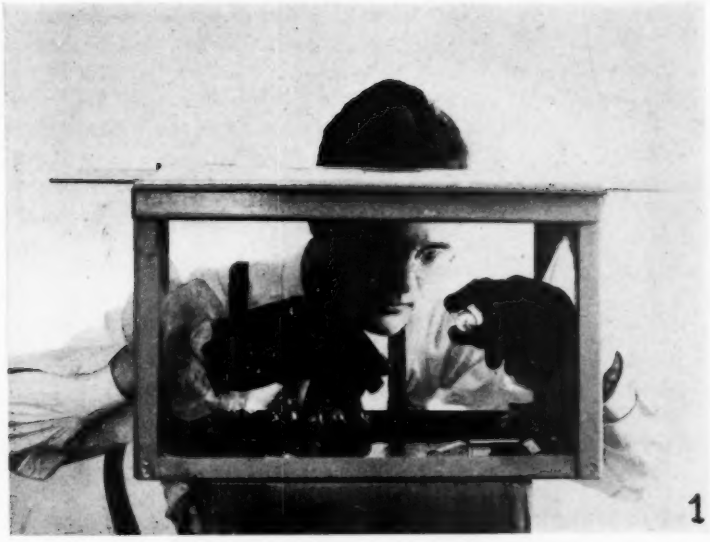

FIG. 1. *Showing resting cage for adult mosquitoes and method of 'loading' vials.*

reactions when extracted. The specimen code number was written immediately below the blood smear. The material was then ready for the serological examination. The feedings were completed in May and June and the laboratory examinations were carried out about three months later.

Serological Procedures

The specific antisera used in this investigation were human, guinea pig, and rabbit. The first two were prepared in the manner described in a previous communication (6), using rabbits as the recipient. Attempts were made to develop an anti-rabbit serum by injecting guinea pigs with rabbit serum. This resulted in sera of low or negligible titre. After several attempts using different routes and regimes of injection this method was abandoned. Cockerels were next used with considerable success and cross reactions did not become troublesome under the conditions of the tests proper, although they were evident when high concentrations of mammalian sera were used.

The setup and method of reading the reactions were identical with that reported in our previous paper. It is worth noting here that the precision of reading is much higher after several hundred tests have been read than when a new series is started, even though the worker may have had considerable experience with the precipitin reaction.

Discussion and Conclusions

The results of this study indicate a high degree of specificity for the precipitin reaction. In a total of 1097 individual feedings (Table II) only 53 errors of all types were observed. The total individual feedings were made up of negative, single, double, and triple feedings. For the purposes of evaluating the tests, each negative and single feeding was considered one feeding, each

TABLE II

SUMMARY OF TOTAL FEEDINGS AND REACTIONS

Feeding	No. specimens	Total individual feedings	Reactions			Total errors
			Correct	Missed	Extra	
Unfed	90*	90	90	—	—	0
Single	307	307	287	13	7	20
Double	341	682	652	26	4	30
Triple	6	18	15	3	—	3
Totals		1097	1044	42	11	53
		Error		4.83%		
		Specificity		95.17%		

** This includes 79 unfed specimens of Culex tarsalis reared from larvae. They gave negative results to human, equine, bovine, and avian antisera.*

double two, and each triple three individual feedings. Thus 341 mosquitoes that had fed on two different hosts provided 682 individual feedings for the purpose of analysis.

Attention is called to the fact that of the 90 negative feedings 11 were from the series using *Aedes aegypti* that were tested against human, rabbit, and guinea pig antisera, while 79 were *Culex tarsalis* Coquillett reared from larvae collected locally and tested against human equine, bovine, and avian antisera. All specimens showed uniformly negative results. This is a small series, but when it is considered that in the total series of 1097 feedings, negative and positive, only 11 extra reactions were noted, it is safe to conclude that false positives occur only in approximately 1% of reactions.

It should be further pointed out that a few of the extra reactions, so-called false positives, might possibly be the result of clerical errors. Although, naturally, every precaution was taken to avoid errors, it must be borne in mind that over one thousand feedings had to be recorded twice; first, during the actual feeding of the specimens, and, second, during the reading of the precipitin reaction.

In recording errors all known feedings that were missed and all incorrect reactions were included. For example, in cases where there was a human and guinea pig feeding and the readings gave human and rabbit as the result, this would be recorded as two errors, one missed and one extra reaction.

From Table II it may be noted that there was a total of 53 errors in 1097 known feedings. This gives a specificity of 95.1%. If the negative feedings are eliminated from that calculation we secure a value of 94.7% sensitivity for positive reactions.

The time of holding the mosquitoes after feeding varied from 5 hr. to 97 hr. at room temperature. There appears to be only a slight decrease in the sensitivity during this time interval and the indication is that in the case of gorged specimens at 97 hr. the number giving a positive reaction is in the neighborhood of 90%. As would be expected, whether a precipitin reaction will occur depends not only upon the time interval between feeding and killing, but also upon the amount of blood taken at the feeding. Thus in the case of double feedings where one of the expected reactions was not obtained it was usually the first feeding that was not detected when the mosquito was gorged at both meals. If gorging occurred at one meal with only a partial feeding at the other it was invariably the latter that failed to show a reaction irrespective of whether it was the first or second feeding.

With the specificity and sensitivity so high it is evident that results of the tests on insects collected in the field must have great significance. Table II shows that from 341 double feedings, equivalent to 682 individual feedings, 656 correct results were obtained. On the basis of double feedings this gives 92.4% of the specimens completely correct. Thus the multiple feedings reported in earlier papers (1, 6) have definite significance. The number of

triple feedings was very small but of the six specimens correct results were secured in three, while in the others, one feeding was missed in each case.

Of added interest and importance as related to field collections is the fact that in 162 specimens out of a total of 654 mosquitoes known to have fed on one or more hosts, the abdomen appeared to be undistended when killed. These specimens represented a total of 217 feedings and of these 201 were correctly determined by the precipitin test. This is equivalent to 91.2% accuracy. It has been the custom in the past (1-6) to use only insects with distended abdomens for these tests. However, it now appears that significant results may be secured by examining a representative sample from any field collection.

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EFFECT OF DIETARY PROTEIN ON SUSCEPTIBILITY OF RATS TO TRAUMA[1]

By C. Gwendoline Toby[2] and R. L. Noble[3]

Abstract

The relation of dietary protein to susceptibility of the rat to trauma has been investigated. Protein levels of from 13 to 18% with a daily intake of 1 to 1.5 gm. appeared to give the greatest protection. Levels of casein, Amigen, and wheat germ protein as low as 8.4% were effective. Protection was afforded by diets too low in adequate protein to support growth, and by the incomplete protein gelatin. Tolerance to trauma on these diets was somewhat decreased if the vitamin B complex was omitted from the diets for two weeks. This was particularly evident at very high levels of protein intake. Body weight changes were unrelated to the tolerance of the rats to trauma. The complete absence of protein from the diet previous to trauma greatly increased the susceptibility of rats to shock. The time required to bring about either increased or decreased tolerance to trauma was much shorter in young than in adult animals. Although stock rats lost their normal tolerance, and resistant rats their acquired resistance to trauma when deprived of protein, it was possible to develop or maintain such resistance if trauma was administered at intervals during the time that the animals were maintained on the protein-free diet.

There has for many years been a great interest in the effect of diet on health and growth, particularly in the quantity and quality of protein required and in the necessity for an adequate amount of the various vitamins. It was noted long ago that certain single proteins, no matter how high the intake, failed to maintain weight or produce growth, whereas others, although inducing growth, were not all equally efficient. This led to an evaluation of proteins according to their biological value. Since many of the component amino acids of various proteins have been isolated, it has been possible to determine which of these are essential for growth in the rat (9) and in man (10, 11) and in what proportions they are most efficient. During the war and before, interest increased in the subject of the negative nitrogen balance that occurs following various types of trauma including surgery, fractures, and burns. Research has centred on postoperative or post-traumatic diets that would prevent or lessen the excessive loss of nitrogen. High protein diets have been advocated by Cuthbertson (2) but Howard (4) and others reported these to have little effect at the height of the protein catabolism. Much use has been made of intravenous feeding with enzymatic digests of casein such as Amigen, and with mixtures of all the essential amino acids. None of these have been entirely successful in arresting the loss of nitrogen or markedly affecting the degree of shock.

It seemed possible that alterations in the diet preceding the subjection of rats to trauma might alter their susceptibility to shock. The initial experiments were done using a modification of a casein diet described by Coward (1).

[1] *Manuscript received July 29, 1947.*

Contribution from the Research Institute of Endocrinology, McGill University, Montreal, Que., with financial assistance from the National Research Council, Ottawa. Some of these results have been reported at the Josiah Macy, Jr. Foundation Conference, 10th meeting, 1945.

[2] *Lecturer.*

[3] *Associate Professor.*

The tolerance to trauma of rats on this diet was remarkably increased over that of rats on the stock diet of Purina fox chow. Hitherto, the tolerance of the normal rat on the stock diet, which contains 24% of mixed protein (19% utilizable) and gives good growth and reproduction, had been considered maximal. This observation led to an extensive series of experiments using different types of protein at different intake levels in attempts to obtain maximal protection. The effects on susceptibility of feeding low levels of protein and protein-free diets, and of withholding vitamins particularly of the B complex were investigated. Following a preliminary report on these results (8, p. 233) Shorr, Zweifach, and Furchgott (13) confirmed the observation that an increased susceptibility to shock followed protein depletion.

Methods

Because of the very large numbers of rats used in these experiments it was not always possible to use animals of the same sex and weight, but for the most part females two to two and one-half months weighing about 150 gm. were used. Trauma was administered in the drum as described previously (7). Resistant rats were made so by repeated subjection to increasing amounts of trauma so that they finally withstood more than the amount ordinarily fatal (6). The stock diet was Purina fox chow. As far as possible, the rats were maintained under standard conditions, six rats to a large cage with a wire-mesh false bottom. It was not possible to maintain a constant room temperature but control groups of animals were run from time to time throughout the experiments with little alteration in mortality.

All the diets were fed in powdered form in low dishes with metal tops that had a hole in the center 1 in. in diameter. This allowed the rats easy access to the food and almost entirely prevented spilling, making it possible to estimate with a fair degree of accuracy the actual food consumption of the group. All the constituents of the diets were first mixed by hand and then by rotation and inversion in closed metal containers to obtain a uniform mixture. Care was taken to keep the diets in the refrigerator and to make up fresh lots at least every two weeks since, if they were kept any length of time—even at low temperatures—some change occurred that lessened the rats' appetites and was rapidly reflected in the weight curves. The composition of the diets used is described in Table I, and each diet will be referred to in the text by its letter–number. The basic diet was composed of protein, fat in the form of lard, carbohydrate as a mixture of cornstarch and glucose, cod liver oil, and vitamin B complex either as yeast or in crystalline form. In the latter case the mixture consisted of thiamin, riboflavin, pyridoxine, calcium panthothenate, and nicotinamide, and was adequate for growth and health. These were given at more than an adequate level (5) particularly thiamin and riboflavin, which were given at 10 times the recommended dose. The mixture amounted to 0.425 mgm. per 10 gm. of diet, which was estimated as approximately the daily intake. This basic diet, with casein as the source of protein, supported good health and growth indefinitely. The proportion of fat in the

diets was kept approximately constant, the carbohydrate portion being varied to adjust the percentage of protein. Although Coward does not include fat in the diets used in vitamin work, other workers suggest that fat increased the digestibility and retention of food nitrogen (3).

Results

Adequate Protein Diets

In previous experiments a quantitative, reproducible relationship was established between the amounts of trauma administered in the Noble–Collip drum, and the percentage mortality resulting (7). The animals used in such experiments were fed the stock diet of Purina fox chow. Further groups fed on this diet were compared throughout the work described here. For the experimental diets described in Table I, casein was first chosen as the main source of protein. Table II shows the effect on mortality from shock of feeding a number of these containing adequate or more than adequate levels of protein. The time required to bring about a change in susceptibility, and the influence of vitamins in the diet is also shown.

When Diet B, containing 12% casein protein and 3% yeast protein (also the source of vitamin B complex) was fed for 7 to 14 days, a marked improvement in the tolerance of rats to drum trauma occurred. After 800 turns, the mortality of casein-fed animals was only 27%, compared with 80% in those fed Purina fox chow. The survival time of those that succumbed was greatly prolonged in the Diet B series. Since yeast was present in small quantity in this diet, it seemed possible that increasing its concentration and thereby that of the total protein might effect further improvement. In Diets B-10 and B-13 the content of casein protein was kept approximately the same (10 and 12%) and the yeast protein was raised to 8 and 20%. Diet B-10, with a total protein count of 18%, was not more effective than Diet B in decreasing the mortality after trauma. When yeast protein was increased to 20%, making the total protein 32% (Diet B-13), the mortality began to rise. When approximately the same total protein content (30%) was achieved by increasing casein protein to 19% (Diet B-19) no improvement was noted. Since increasing yeast protein, or total protein, was not attended by any increase in tolerance over that provided by Diet B, it was decided to investigate diets in which casein was the sôle source of protein, and vitamins, when added, were supplied in crystalline form. Diet B-4, with a protein content of 13%, contained no added vitamins. In the groups fed this diet, the food intake was normal, but growth was retarded during the two-week period; no other sign of vitamin deficiency was evident. These animals, when subjected to 500 turns in the drum, appeared to be as well protected as rats on Diet B, but when trauma was raised to 800 turns, greater susceptibility was evident. When this diet was fortified with vitamins (Diet B-5), the food intake was about the same, but growth was better and the rats showed no mortality after 500 turns and only 9% after 800 turns.

TABLE I

DESCRIPTION OF DIETS

Diet	Protein		CHO	Fat	Salts	Added vitamins	
	Source and amount	% in diet				C.L. oil	B complex
A	Purina fox chow	24	(48)	(4.7)	(5.6)		
B	Casein 15, yeast 7.5	15	66	6	4	+	−
B- 4	Casein 15	13	70	6	4	+	−
B- 5	Casein 15	13	70	6	4	+	+
B- 6	Casein 5.7	4.5	80	6	4	+	+
B- 7	Casein 11	8.4	76	6	4	+	+
B-10	Casein 13, yeast 20	18	57	6	4	+	−
B-11	Casein 1.2	0.9	85	6	4	+	+
B-13	Casein 16, yeast 48	32	24	6	4	+	−
B-19	Casein 24, yeast 34	30	30	6	4	+	−
C	None	0.10	88	8	4	−	−
C- 1	None	0.10	88	8	4	+	+
C- 2	None	0.10	88	8	4	+	−
C- 3	None	0.10	100	0	0	−	−
F	Casein 100	77	0	0	0	−	−
F- 1	Casein 80	60	20	0	0	−	−
F- 2	Casein 40	30	60	0	0	−	+
F- 3	Casein 40	30	60	0	0	−	−
F- 4	Casein 100	77	0	0	0	−	+
F- 5	Casein 80	60	20	0	0	−	+
G- 7	Gelatin 11	10	77	6	4	+	+
G- 9	Gelatin 20	18	70	6	4	+	+
H	Dried skimmed milk 16	5.5	80	0	0	+	+
H- 1	Dried skimmed milk 50	17	48	0	0	+	+
M- 2	Tonik germ 20	8	70	6	4	+	+
P	Lactalbumin digest (Ayerst) 13.8	10	76	6	4	+	+
Q	Amino acid powder (Ayerst)	10	68	6	4	+	+
Z- 7	Zein 10	9	79	6	4	+	+

Explanatory notes:

Amounts are given as grams per 100 gm. of diet (approximate).

Carbohydrate (CHO)—mixture of corn starch and glucose, 5 : 1.

Fat—lard.

Salts—Steinbock's salt mixture No. 40.

Vitamins added—cod liver oil 2 parts/100, vitamin B complex 4.25 mgm./100 gm. diet.

Purina fox chow—the data are taken from the commercial pamphlet. The protein, checked by nitrogen analysis, is 24% of which 19% is said to be utilizable. The remainder of the data is given in parentheses.

Protein values given are based on nitrogen analysis assuming the nitrogen content to be 16% except in the case of gelatin (18%). Each new lot was checked before using.

Casein—obtained from Bulldog Grip Cement Co. Protein 75 to 77.5%.

Casec—calcium caseinate—Mead Johnson & Co. Protein 83.7%.

Yeast—National Breweries Ltd. Protein 40 to 40.8%.

Gelatin—confectioner's—Rose & LaFlamme Ltd. Protein 92.4%.

Dried skimmed milk—The Borden Company, Ltd. Protein 34%.

Amigen—Mead Johnson & Co. Protein 75%. (Stated to be 50% hydrolyzed).

Wheat germ protein—prepared by Ogilvie Flour Mills Co., Ltd., from defatted wheat germ. Protein 80%.

Tonik wheat germ—partially defatted preparation of above. Protein 40%.

Zein—(corn) obtained from Pfanstiehl Chemical Co. Protein 90%.

Lactalbumin (Ayerst) supplied for experimental purposes. Protein 72.3%.

Amino acid powder (Ayerst) supplied for experimental purposes. Protein 45%.

TABLE II

EFFECT OF CASEIN DIETS ON MORTALITY AFTER TRAUMA

Sex of rats	Diet			Average food intake, gm./rat/day	No. days on diet	Average total wt. gain, gm./rat	% Mortality after	
	No.	Source of protein	Protein, %				500 turns	800 turns
M	A	Purina	24	14-16	—	—	48 (48)*	97 (34)
F	A	Purina	24	—	—	—	35 (30)	80 (40)
F	B	Casein 12%, yeast 3%	15	12-14	1-2	—	—	58 (16)
F	B	Casein 12%, yeast 3%	15	12-14	3	8	50 (4)	25 (8)
F	B	Casein 12%, yeast 3%	15	12-14	7	17	25 (4)	25 (8)
F	B	Casein 12%, yeast 3%	15	12-14	14	28	12 (8)	27 (11)
F	B-10	Casein 10%, yeast 8%	18	—	14	14	8 (12)	—
M	B-10	Casein 10%, yeast 8%	18	—	14	36	—	55 (24)
M	B-19	Casein 19%, yeast 11%	30	—	14	35	33 (6)	83 (6)
F	B-13	Casein 12%, yeast 20%	32	—	14	21	—	45 (11)
F	B-5	Casein (+ crystalline vitamins)	13	11	14	16	0 (10)	9 (11)
F	B-4	Casein (no vitamins)	13	12	14	6	9 (11)	50 (12)
F	F-2	Casein (+ vitamins)	30	14	14	−2	17 (12)	—
M	F-2	Casein (+ vitamins)	30	15	14	18	—	58 (18)
M	F-3	Casein (no vitamins)	30	15	14	0	—	75 (12)
M	F-5	Casein (+ vitamins)	60	14	14	7	25 (12)	—
F	F-1	Casein (no vitamins)	60	10	14	−1	58 (12)	—
M	F-4	Casein (+ vitamins)	77	12	14	−19	90 (11)	—
F	F	Casein (no vitamins)	77	10	14	−6	83 (12)	—

* *Number of rats given in parentheses.*

In view of these results, diets of higher casein content were tested (F to F-5) with and without added vitamins. These diets contained no fat or salt mixture, since it seemed desirable to maintain as high a carbohydrate content as possible. Both males and females, fed these casein diets, showed somewhat greater tolerance to trauma than did rats fed the same amount of mixed protein. When vitamins were not added to the diet (F-3) mortality after trauma increased. The amount of protection afforded by 60% protein with vitamins (F-5) was the same as that given by 30% and although food consumption remained almost the same, growth on the higher intake practically ceased. When this diet did not include any vitamins (F-1) although the food intake was 10 gm. per day, mortality rose sharply. At protein levels of 77%, the food intake was 12 gm. per day but weight loss occurred even when vitamins were present in the diet and mortality after 500 turns rose to almost 100%.

Diets Containing Low Levels of Complete Protein

Since casein diets over a wide range of protein concentration, in many instances lower than Purina fox chow, afforded excellent protection against trauma, it seemed of interest to determine how little protein would be adequate in maintaining normal tolerance. Table III shows the results of these experiments. Three diets containing only casein as a source of protein were used: B-11, B-6, and B-7, containing, respectively 0.9, 4.5, and 8.4% protein. Although in this series food intake was not measured in all cases, the animals ate well, but on the two lower levels of protein intake lost weight over the two-week period. On 8.4% protein, the rats grew slowly. Mortality after 500 turns was 100% on 0.9% protein, and decreased sharply with increasing protein concentration. Diet B-7 containing 8.4% casein protein afforded the same protection (33% mortality) as Purina fox chow, containing 24% of mixed protein. Wheat germ protein (Diet M-2) was compared with casein. At a level of 8% protein, 10 gm. per day of the diet was eaten and this amount allowed female rats to maintain their initial weight. Their tolerance to trauma compared favorably with that of rats fed the same concentration of casein protein. A group of male rats fed this diet (M-2) for 28 days ate on an average 14 gm. per day and gained 36 gm. in this time. Their mortality following trauma was also remarkably low.

TABLE III

EFFECT OF LOW LEVELS OF PROTEIN ON MORTALITY AFTER TRAUMA

Sex of rats	Diet for two weeks			Average food intake, gm./rat/day	Average total wt. change, gm./rat	% Mortality after	
	No.	Source of protein	Protein, %			500 turns	800 turns
F	B-11	Casein	0.9	—	−18	100 (12)*	—
F	B- 6	Casein	4.5	—	− 4	50 (24)	—
F	B- 7	Casein	8.4	—	+12	33 (12)	—
F	M- 2	Wheat germ	8.0	10	+ 4	—	50 (6)
F	M- 2	Wheat germ	8.0	11	− 3	20 (6)	—
M	M- 2	Wheat germ	8.0	14	+36 (28 days)	33 (6)	—
F	H	Skimmed milk	5.5	—	− 9	42 (12)	—
M	H- 1	Skimmed milk	17.0	16	− 3	—	83 (6)

* *Number of rats given in parentheses.*

Dried skimmed milk diets (H and H-1) at protein levels of 5.5 and 17% gave approximately the same protection as Purina fox chow, although diarrhoea occurred and the animals lost some weight.

Diets Containing Incomplete Protein and No Protein

In view of the critical level of protein demonstrated to be necessary to afford protection against trauma, diets containing no protein or an incomplete protein (gelatin) were fed in order to study the response of such depleted

animals to trauma. After seven days on the protein-free diet (Diet C) the mortality in female rats increased to 55% after 500 turns in the drum, and at nine days 300 turns was fatal to 40% of a group of male rats. Two weeks of protein depletion raised the mortality consistently to 84% after 500 turns in groups of females totalling 156 animals. When groups of animals were fed Diet C for 9 to 22 days, and then subjected to trauma of 800 turns, the mortality rose to nearly 100% by the ninth day and continued at this high level. Table IV contains the results of some of these experiments. The presence of fat, salt mixture, or vitamins had little effect (Diets C-1, C-2, C-3).

TABLE IV

EFFECT OF FEEDING OF PROTEIN-FREE OR INCOMPLETE PROTEIN DIETS ON MORTALITY AFTER TRAUMA

Sex of rats	Diet for two weeks			Average food intake, gm./rat/day	Average total wt.change, gm./rat	% Mortality after	
	No.	Source of protein	Protein, %			500 turns	800 turns
F	C	None	—	8	−28	84 (156)*	83 (12)
M	C	None	—	9	—	—	100 (19)
F	C	None (21 days on diet)	—	—	−38	—	100 (20)
F	C-1	C + vitamins	—	—	—	75 (12)	—
F	C-2	C + cod liver oil	—	—	—	91 (11)	—
F	C-3	Carbohydrate only	—	—	—	91 (11)	—
F	G-7	Gelatin	10.0	9	−26	27 (36)	—
F	G-9	Gelatin	18.0	7	−26	40 (10)	—

**Number of rats given in parentheses.*

When groups of rats were fed a diet containing gelatin as the only source of protein, the weight loss (26 gm.) was as great as in many of the groups fed Diet C. This diet (G-7) in spite of a lower intake, afforded better protection against trauma than did Purina fox chow, and as good protection as many of the casein diets.

Effect of Depletion of Protein and of Various Levels of Protein Intake on Mortality in Young Male Rats Following Trauma

In this series the effect of deprivation of protein and of feeding different types and amounts of protein to young rats was investigated. None of the proteins was supplied in sufficiently high concentration to allow maximum growth, with the exception of the 20% casein protein diet, on which growth was slightly better than on Purina fox chow. In this series male rats weighing 80 to 100 gm. and from one to one and one-half months old were used. On the stock diet half the animals died following 500 turns in the drum, and all died following 800 turns. Deprivation of protein rapidly increased the susceptibility to shock in these young animals; five days on Diet C was sufficient to increase mortality to 42% at 400 turns, and to 100% at 500 turns.

Amigen, casein, and wheat germ were fed at various levels and gelatin and several other proteins not previously tested in adult rats were also used. The average amount of protein consumed each day was estimated, and the results are evaluated on that basis in Table V.

TABLE V

EFFECTS OF DIFFERENT TYPES AND AMOUNTS OF PROTEIN ON MORTALITY OF YOUNG RATS FOLLOWING TRAUMA

Type of protein in diet	Average protein intake, gm./rat/day	Days on diet	Average total wt. change, gm./rat	% Mortality after	
				400 turns	500 turns
Purina	2.16	14	+41	—	50 (6)*
None	0	5	−12	—	100 (18)
None	0	5-7	−14	42 (12)	—
Casein	0.60	5	0	—	66 (6)
Casein	0.88	5	+ 8	—	100 (6)
Casein	0.96	14	+23	—	83 (6)
Casein	1.25	5	+11	—	33 (6)
Casein	1.48	5	+10	—	20 (6)
Casein	1.82	7	+25	—	33 (6)
Casein	1.82	7	+25	0 (6)	—
Amigen	0.93	5	+ 9	—	100 (6)
Amigen	1.04	5	+ 8	—	50 (6)
Amigen	1.09	14	+34	—	50 (6)
Amigen after mild acid hydrolysis	0.91	5	+ 4	—	66 (6)
Amigen after mild acid hydrolysis	0.93	5	+ 5	—	33 (6)
Tonik wheat germ	0.64	14	− 8	—	100 (6)
Tonik wheat germ	0.64	14	− 8	83 (6)	—
Tonik wheat germ	0.78	5	+ 1	—	66 (6)
Tonik wheat germ	0.88	21	+23	25 (4)	—
Tonik wheat germ	1.10	14	+26	—	100 (6)
Lactalbumin digest (Ayerst)	0.91	14	+20	—	50 (6)
Amino acid powder (casein digest) (Ayerst)	1.02	14	+26	—	83 (6)
Gelatin	0.70	5	−16	—	83 (6)
Gelatin after mild hydrolysis	1.08	5	−12	—	66 (6)
Zein	0.65	5	−12	—	66 (6)

* *Number of animals given in parentheses.*

With only two exceptions, increasing intake of a given protein was paralleled by increasing tolerance to trauma. This relation was well demonstrated, in spite of the small number of animals in each group. The only inconsistent value in the casein series was in the group in which the protein intake was lowest (0.60 gm. per day), and here the protection was greater than could be

expected on the basis of the rest of the results. With increasing intakes from 0.88 gm. to 1.48 gm. per day, the mortality diminished from 100 to 20%. No further improvement was seen when the amount of protein was increased to 1.82 gm.

Amigen, of which about 50% is stated to be hydrolyzed to amino acids, gave approximately the same results as casein. Mild acid hydrolysis of Amigen apparently partially destroyed the tryptophan present, since growth on this preparation was only about one-half that on the original Amigen. Protection, however, seemed to increase after the further hydrolysis.

'Tonik' Wheat Germ gave almost as good protection in the same range as casein and Amigen, with the exception of one experiment. In the group that had the highest average daily intake of protein, the concentration of protein in the diet was higher and for the first week the animals ate very much less, with only a slight gain in weight (6 gm.). In contrast, the weight increase in the second week was great (20 gm.). The digest of lactalbumin gave good growth and protection, less than 1 gm. per day protecting half the rats against death from shock.

Gelatin when hydrolyzed was apparently more palatable than the unhydrolyzed substance since the rats consumed an amount of food containing an average of 1.08 gm. of this per day compared with 0.7 gm. of the original material. At the higher intake level, it compared well with casein and Amigen in affording protection against shock.

Zein also gave good protection in the low amount of 0.65 gm. per day.

In some cases these comparisons are necessarily approximate, since food was given ad libitum and the protein intake calculated from the amount eaten. The value given is the average intake for the group.

Five days on a protein-free diet was sufficient to increase the mortality after 500 turns to 100%. Similarly five days on the experimental protein diets were sufficient to give the maximum improvement in protection.

Effect of Diet on Resistance and on the Development of Resistance in Normal Animals

Effect of Protein Depletion

When 16 trauma resistant rats that had been subjected to 1000 turns in the drum repeatedly were placed on a protein-free diet for two or three weeks, the mortality following 1000 turns rose to 50% in each case. Restoration of such animals to the stock diet following three weeks on the protein-free diet resulted in the complete return of resistance. If, however, resistant animals were subjected to trauma at intervals during a two-week period on Diet C, they maintained their normal resistance and no mortality resulted after 1000 turns.

Normal rats, both male and female, were made resistant while on a protein-free diet. They were kept on this diet for two weeks before receiving any trauma and were then traumatized an increasing number of turns at intervals of two to three days over a period of another 12 to 14 days. Of 29 rats, 14

survived 1000 turns. Some of the casualties occurred after small amounts of trauma. Of those finally receiving 1000 turns, only one died. If care was taken to increase the amount of trauma more gradually than is necessary in protein-fed animals, it was possible for depleted rats to become resistant to repeated trauma of 1000 turns, even after complete absence of protein from the diet for a period of 28 days.

Effect of Feeding Gelatin

Four resistant female rats maintained on the gelatin-containing diet, G-7, for two weeks survived 1200 turns in the drum, while of four more maintained on the diet for three weeks, one died eight hours after receiving the trauma. The rats received no trauma during the two or three weeks on this diet, but neverthless retained their resistance in spite of considerable depletion of body protein.

Discussion

In the experiments described it was found that the tolerance of rats to trauma is related to the amount and type of protein in the diets. Casein, wheat germ, gelatin, gelatin digests, lactalbumin digests, and Amigen were particularly effective in affording protection. Improvement in the tolerance of adult rats was noted as early as the third day, and was maximum after 7 to 14 days on the diets. Levels of casein and wheat germ protein that did not support growth nevertheless imparted considerable protection against trauma. With levels of 12 to 15% casein protein in the diet, mortality, which on Purina fox chow was almost 100% after 800 turns in the drum, was reduced to less than 25%. The acid hydrolysis and apparent destruction of tryptophan did not decrease the protective effect of Amigen. Gelatin as the sole source of protein in the diet conferred a high degree of protection, although lacking in tryptophan, valine, and methionine, and resulting in as great a weight loss as occurred in animals completely deprived of protein.

The fact that tolerance to trauma did not increase with casein levels above 40% (30% protein) and actually decreased with higher levels may be due in part to the necessary reduction in carbohydrate. The stock diet of Purina fox chow, which contains only 48% carbohydrate, was found to be somewhat improved when glucose was added to raise the level to 63%. Yeast protein above 8% did not improve the casein diets. Lack of vitamins for two weeks did not greatly increase the susceptibility until the protein content of the diet exceeded 30%.

There appeared to be no direct relation between gain or loss in weight and changes in susceptibility to trauma. Complete absence of protein from the diet for two weeks previous to trauma greatly increased the susceptibility of rats to shock. Young rats became extremely susceptible after five days on protein-free diets, and improved their tolerance somewhat more rapidly than adults when placed on protective diets.

Lack of dietary protein caused trauma resistant animals to lose their acquired resistance within two weeks whereas on a normal diet such resistance, once fully developed, lasts for a period of several months without further

stimulus (6). Gelatin diets were as effective as adequate diets in maintaining resistance. When animals that had lost their resistance on a protein-free diet were again placed on the stock diet, their acquired resistance was fully restored. Resistance was maintained on a protein-free diet if the animals were subjected to trauma during the period of protein depletion. In addition, normal rats previously deprived of protein for two weeks could be made resistant while continuing on this diet for as long as 28 days. The possibility that certain amino acids might be specifically involved in the protective mechanism will be reported in a following paper.

It would seem that suitable dietary protein provides some material that affords protection against shock from drum trauma and that such material is also necessary for the maintenance of acquired resistance. Its action may be similar in nature to the replacement of antibody protein, which has been shown to be replenished from the protein of the diet (12). Since resistance can, however, be developed or maintained in the absence of dietary protein, the body must, under the stimulus of trauma, be able to provide the required material from its own protein stores. It would appear that the body, in response to trauma, conserves or makes available some substance required for the constant rebuilding of protective material. In the protein-depleted animal it may be necessary to transfer amino acids from body stores for this rebuilding whereas, in the normally fed animal, the dietary protein supplies the requirement. Lowered tissue stores and the needs of essential organs may prevent this material being readily available after protein depletion.

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INFLUENCE OF REPLACEMENT THERAPY ON THE INCREASED SUSCEPTIBILITY OF PROTEIN-DEFICIENT RATS TO TRAUMA[1]

By C. Gwendoline Toby[2] and R. L. Noble[3]

Abstract

Rats, following periods of protein depletion, showed a decreased tolerance to trauma. Protein feeding restored normal tolerance but five or six days feeding was necessary to correct two weeks' depletion. The amount of protein consumed was an important factor when low levels of 0.08 to 0.68 gm. per day were used. Casein, Amigen, and wheat germ protein were effective for replacement. After protein depletion of one month or more, two or three weeks of protein feeding was required to restore normal tolerance. No individual amino acid was found to afford protection against trauma although a mixture of the essential amino acids in one case had some effect. Gelatin was an effective protein for replacement therapy. In a single experiment in which a mixture of glutamic acid and glycine of the same total nitrogen content as gelatin was administered, the same degree of protection was afforded as by gelatin.

In the preceding paper, studies on the effect of protein depletion on the tolerance of rats to trauma have been reported (3). Animals deprived of protein were found to be much more susceptible to trauma. In the present paper the effectiveness of adequate protein diets in restoring normal tolerance after varying lengths of time of depletion has been investigated. In addition, experiments have been done in which single amino acids or groups of amino acids were added to the protein-free diet to determine their effect on the susceptibility of rats following trauma.

Methods

Black hooded rats reared in the laboratory, mostly females two to three months old, were used. The stock diet was Purina fox chow and the experimental diets used are listed in Table I. Details of the preparation and feeding of the diets, and the method of applying trauma have been described (3, 2). The food consumption of each cage of four or six rats was measured and the average daily intake of protein per rat calculated. In experiments in which a definite daily intake of the material to be tested was desired, it was mixed each day with an amount of the basal diet determined by the previous day's consumption. Amino acids were tested by incorporating them into both adequate and protein-free diets. Vuj,* a mixture of all the essential amino acids in the following proportions was also tested.

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* *Kindly supplied by Merck Chemical Company.*

TABLE I

DESCRIPTION OF DIETS

Diet	Protein		CHO	Fat	Salts	Added vitamins	
	Source and amount	% in diet				C. L. oil	B complex
A	Purina fox chow	24	(48)	(4.7)	(5.6)		
B	Casein 15, yeast 7.5	15	66	6	4	+	−
B- 5	Casein 15	13	70	6	4	+	+
B- 6	Casein 5.7	4.5	80	6	4	+	+
B-11	Casein 1.2	0.9	85	6	4	+	+
B-12	Casein 3.5	2.7	85	6	4	+	+
B-14	Casein 32, yeast 8	28	48	6	4	+	−
B-16	Casec 32, yeast 8	30	48	6	4	+	−
B-17	Casein 13	10	75	6	4	+	+
B-18	Casein 26	20	62	6	4	+	+
C	None	0.10	88	8	4	−	−
C- 1	None	0.10	88	8	4	+	+
C- 4	None	0.10	88	8	4	−	+
F- 2	Casein 40	30	60	0	0	−	+
G- 2	Casein 16, gelatin 12	23	60	6	4	+	+
G- 7	Gelatin 11	10	77	6	4	+	+
H- 2	Dried skimmed milk 36, casein 16	24	36	6	4	+	+
K- 1	Amigen 3	2.25	86	7	4	−	+
K- 2	Amigen 5	3.8	85	6	4	−	+
K- 3	Amigen 10	7.5	80	6	4	−	+
K- 4	Amigen 15	11	75	6	4	−	+
K- 5	Amigen 20	15	70	6	4	−	+
L- 1	Wheat germ 10	8.2	79	6	4	−	+
L- 2	Wheat germ 20	16.4	70	6	4	−	+
M- 1	Tonik germ 20	8	70	6	4	−	+
M- 3	Tonik germ 40	16	50	6	4	−	+
M- 4	Tonik germ 10	4	80	6	4	−	+
Z- 2	Casein 16, zein 12	23	67	6	4	+	+

Explanatory notes:

Amounts are given as grams per 100 gm. of diet (approximate).

Carbohydrate (CHO)—mixture of corn starch and glucose 5 : 1.

Fat—lard.

Salts—Steinbock's salt mixture No. 40.

Vitamins added—cod liver oil 2 parts /100.

vitamin B complex 4.25 mgm./100 gm. diet.

Purina fox chow—the data is taken from the commercial pamphlet.

The protein, checked by nitrogen analysis, is 24% of which 19% is said to be utilizable. The remainder of the data is given in parentheses.

Protein values given are based on nitrogen analysis assuming the nitrogen content to be 16% except in the case of gelatin (18%). Each new lot was checked before using.

Casein—obtained from Bulldog Grip Cement Co. Protein 75 to 77.5%.

Casec—calcium caseinate—Mead Johnson & Co. Protein 83.7%.

Yeast—National Breweries Ltd. Protein 40 to 40.8%.

Gelatin—confectioner's—Rose & LaFlamme Ltd. Protein 92.4%.

Dried skimmed milk—The Borden Company Ltd. Protein 34%.

Amigen—Mead Johnson & Co. Protein 75% (stated to be 50% hydrolyzed).

Wheat germ protein—prepared by Ogilvie Flour Mills Co., Ltd., from defatted whea germ. Protein 80%.

Tonik wheat germ—partially defatted preparation of above. Protein 40%.

Zein—(corn) obtained from Pfanstiehl Chemical Co. Protein 90%.

dl-Threonine	10.77 gm.
dl-Valine	13.85 "
l (−) Leucine	15.38 "
dl-Isoleucine	10.77 "
l (+) Lysine hydrochloride	12.31 "
dl-Tryptophan	1.85 "
dl-Phenylalanine	6.92 "
dl-Methionine	6.15 "
l (+) Histidine hydrochloride	4.00 "
l (+) Arginine hydrochloride	8.00 "
Glycine	10.00 "
	100.00 gm.

Results

Effect of Length of Time of Replacement and Percentage of Protein on Mortality Following Trauma

In the first series of experiments, following two weeks of protein depletion, rats were fed adequate diets containing different concentrations of protein from 13 to 30%. Food consumption was estimated to be approximately 12 to 14 gm. per rat per day and weight increase was rapid. Trauma was administered after two to six days of protein feeding. The results are seen in Table II.

For comparative purposes it may be noted that animals that had not been on a deficient diet but were maintained on the stock diet (A) showed 35 to 50% and on Diet B no mortality after 500 turns in the drum. After two weeks on Diet C, mortality in female rats after 500 turns was 85%. The feeding of Purina (Diet A) or Diet B for two days after protein depletion had little effect in reducing mortality. Five days produced a considerable increase in tolerance to trauma, whereas six days produced maximal protection against 500 turns. In most instances the difference in effect between five and six days' replacement was quite marked. When the experimental diets were fed for five days, Diets B-14, B-16, and F-2, containing higher levels of casein protein (28 to 30%) were more effective than Diets B or B-5, which contained only 15 and 13%. After six days, however, the lower levels of protein appeared equally effective. Diet G-2, which contained a mixture of casein and gelatin was as effective as casein alone. Skimmed milk (Diet H-2) added to casein did not increase the protection against trauma.

The increased tolerance occurring with protein feeding did not seem to be directly related to weight gain, although in most cases the animals nearly recovered the weight lost while on the protein-free diet (33 to 39 gm.).

TABLE II

EFFECT OF PROTEIN REPLACEMENT ON TOLERANCE TO TRAUMA OF RATS PREVIOUSLY DEPLETED OF PROTEIN FOR TWO WEEKS

Sex of rats	Diet	% Protein in diet	Days on diet	Average weight gain, gm./rat/day	% Mortality after 500 turns
M	A	24	(Controls – no depletion)		50 (30)*
F	A	24	(Controls – no depletion)		35 (30)
F	B	15	(Controls – no depletion)		0 (30)
M	B	15	(Controls – no depletion)		0 (12)
F	C	0	Controls		85 (156)
F	A	24	2	8.0	75 (4)
F	B	15	2	5.0	100 (4)
F	B	15	5	7.0	57 (15)
F	B	15	6	5.0	25 (4)
F	B- 5	13	5	4.6	60 (6)
F	B- 5	13	6	5.3	0 (4)
M	B-14	28	5	7.2	30 (10)
F	B-14	28	5	5.2	8 (12)
F	B-14	28	6	6.8	0 (4)
M	B-16	30	5	7.6	45 (11)
F	F-2	30	6	3.0	20 (6)
M	G- 2	23	5	6.2	25 (12)
M	H- 2	24	5	5.0	55 (11)

* *Number of rats given in parentheses.*

Effect of Normal Protein Intake After Depletion

In a second series of experiments, three diets containing different proteins at concentrations between 10 and 20% were fed for five to seven days. In this series food consumption was measured and protein intake calculated. These results are shown in Table III. After two weeks' protein depletion, Diets B-17 and B-18 were fed for five, six, and seven days. In each case, increased tolerance was evident with increasing time of replacement and with increasing protein consumption. Time rather than actual daily protein intake appeared to be more important. The daily intake figure was calculated from the total intake over the replacement period, but in most instances the rats ate more when first returned to an adequate diet. Diets containing 10% casein protein were as effective in conferring protection as a diet containing 20% protein. Diet K-5, containing 20% Amigen, appeared to cause the greatest reduction in susceptibility to trauma. An intake of about 1.5 gm. protein per day appeared to give the greatest amount of protection.

TABLE III

EFFECT OF PROTEIN INTAKE ON TOLERANCE OF RATS PREVIOUSLY DEPLETED OF PROTEIN FOR TWO WEEKS

Diet	Days on diet	% Protein in diet	Average protein intake, gm./rat/day	Average weight gain, gm./rat/day	% Mortality after 600 turns
B-17	5	10	1.28	5.4	100 (5)*
B-17	5	10	1.34	5.6	83 (6)
B-17	6	10	1.14	3.5	80 (5)
B-17	7	10	1.10	3.7	66 (6)
B-17	7	10	1.14	3.7	33 (6)
B-18	5	20	2.06	4.8	100 (3)
B-18	5	20	2.10	5.8	66 (6)
B-18	5	20	2.16	4.6	60 (5)
B-18	6	20	2.10	3.5	83 (6)
B-18	7	20	2.20	4.7	50 (6)
B-18	7	20	2.36	4.7	50 (6)
K-5	5	15	1.66	5.0	50 (6)
K-5	5	15	1.83	5.8	50 (6)
K-5	7	15	1.56	4.0	17 (6)
K-5	7	15	1.60	4.7	17 (6)
L-2	5	16	1.97	5.2	50 (6)
L-2	7	16	2.08	4.9	33 (6)

* *Number of rats given in parentheses.*

Effect of Low Levels of Protein Intake After Depletion

In a third series, the depletion period was shortened to nine days. Protein was replaced in the diet for five days before the animals were traumatized. A wider variation in protein intake was used in an attempt to demonstrate more clearly a relation between the amount of dietary protein and the degree of restoration of tolerance. Diets containing casein protein from 0.9 to 20%, Amigen from 2.2 to 15%, and wheat germ from 4 to 16.4% were compared. Table IV contains these results. The average weight losses in Diet C in these groups were more variable—from 23 to 36 gm. over the nine-day period.

With the feeding of increasing amounts of casein protein from 0.08 gm. to 0.68 gm. per day the mortality after 500 turns fell from 66 to 20%. When the intake of protein rose to 1.25 to 1.40 gm. the mortality after 800 turns averaged 58%. When the protein intake doubled, there was little further improvement. Casein and Amigen gave approximately equal protection over a range of 0.25 gm. to 1.40 gm. per day. When the level of protein in the diet reached 1 gm. per day the trauma was increased to 800 turns in the drum, but above 1.4 gm. very little increase in protection was evident. With the more severe trauma maximal protection was not obtained in any instance when the replacement time was restricted to five days. In this series, casein, Amigen, and wheat germ protein all were equally effective in improving the tolerance of protein-depleted rats.

TABLE IV

EFFECT OF REPLACEMENT WITH DIFFERENT TYPES AND LEVELS OF PROTEIN OVER A FIVE-DAY PERIOD AFTER NINE DAYS OF PROTEIN DEPLETION

Diet	% Protein in diet	Protein intake, gm./rat/day	Average weight gain, gm./rat/day	% Mortality after	
				500 Turns	800 Turns
B-11	0.9	0.08	−1.2	66 (6)*	—
B-12	2.7	0.25	0.4	60 (5)	—
B- 6	4.5	0.39	−0.4	50 (6)	—
B- 6	4.5	0.42	−0.4	—	100 (6)
B- 6	4.5	0.68	2.8	20 (5)	—
B-17	10.0	1.25	2.8	—	50 (6)
B-17	10.0	1.40	4.4	—	66 (6)
B-18	20.0	2.20	3.4	—	40 (5)
B-18	20.0	2.44	6.0	—	57 (7)
K-1	2.2	0.26	−0.2	61 (11)	—
K-2	3.8	0.38	0.8	56 (9)	—
K-3	7.5	1.00	2.4	—	100 (9)
K-4	11.2	1.20	2.8	—	100 (5)
K-4	11.2	1.30	3.4	—	43 (6)
K-5	15.0	1.77	3.8	—	83 (6)
K-5	15.0	2.00	5.4	—	50 (6)
L-1	8.2	1.13	3.8	—	91 (11)
L-2	16.4	2.06	5.4	—	66 (6)
M-4	4.0	0.47	0.2	20 (5)	—
M-1	8.0	1.05	2.8	—	83 (6)
M-3	16.0	1.84	4.8	—	92 (12)

* *Number of rats given in parentheses.*

Length of Time Required on a Protein Diet to Restore Normal Tolerance in Rats Depleted of Protein for Prolonged Periods

A few groups of male rats two months old were maintained on Diet C for from 32 to 38 days and then returned to Diet B to determine whether it was possible to restore normal tolerance after this extended deprivation of protein and, if so, how long it would take. The results are shown in Table V.

TABLE V

EFFECT OF PROTEIN REPLACEMENT AFTER PROLONGED PROTEIN DEPLETION

Diet C		Diet B		% Mortality after 500 turns
No. days fed	Average total weight loss, gm./rat	No. days fed	Average total weight gain, gm./rat	
38	86	8	41	100 (6)*
36	90	7	57	83 (6)
32	77	9	65	66 (6)
35	70	14	97	33 (6)
32	57	21	82	0 (6)
32	85	21	120	0 (3)

* *Number of rats given in parentheses.*

It is evident that even after more than a month's deprivation of dietary protein, restoration of protein for two weeks will decrease the animals' high susceptibility to trauma. Growth rate or ingestion of a given amount of protein per day was no indication of increased protection. When the restoration of weight was greater than the loss, and the growth rate decreased, then the animals' tolerance to trauma increased sharply. After 21 days on a casein diet, the rats surpassed their initial weight and were able to withstand 500 turns with no mortality, indicating no permanent effect of the long protein deficiency on their ability to develop an increased tolerance when fed a casein diet.

Effect of Zein and Gelatin When Used as Replacement Therapy

A group of 18 animals that had been on Diet C 9 to 11 days were given diets containing either 10 or 20% zein as the source of protein for five days. With protein intakes of from 0.8 to 1.36 gm. per day the mortality after 500 turns was 33 to 66% compared with that of 100% in control groups without protein. A diet containing 10% gelatin was given for seven days to rats depleted of protein for only seven days. The protein intake averaged 0.53 gm. per day. Of 44 rats so treated the mortality after 500 turns was 33% compared with 86% for 59 untreated animals. An attempt was made to separate hydrolyzed gelatin by butyl alcohol extraction as described by Dakin (1). When the fractions were tested as replacement therapy, there was no evidence that any concentration of activity had been effected. In fact either gelatin or Amigen after 24-hr. hydrolysis by 30% sulphuric acid appeared to have a reduced protective action.

Effect of Amino Acids When Used as Replacement Therapy

In preliminary experiments individual amino acids were added to a basal diet containing 13% casein protein and this was used for five days as a replacement diet after two weeks' protein depletion. The animals then received 600 turns in the drum. Small numbers of rats had to be used in this series and the results obtained were not clear-cut because of some variation in the control animals. However, the greatest protection was shown by methionine; phenylalanine, α-alanine, and arginine had a smaller effect. Choline and creatine were also tested and gave results comparable to arginine. Since the number of animals used was small, it was thought advisable in subsequent experiments to add the amino acids to a protein-free diet containing vitamin B complex (Diet C-4). The addition of vitamins to Diet C did not influence mortality after trauma. The following table (VI) lists the substances that were given and the average amount taken by each animal per day. Mortality was determined after 500 turns and in some cases after 800 turns.

It may be seen that no individual amino acid was effective in reducing the mortality after trauma. In some cases where one appeared to do so after 500 turns, a repetition of the experiment using increased trauma of 800 turns did not indicate protection. Similarly, mixtures of amino acids were not effective. In other experiments a mixture of all the essential amino acids—Vuj (Merck)—

TABLE VI

EFFECT OF AMINO ACIDS ADDED TO A PROTEIN-FREE DIET

Substance added to Diet C-4	Amino acid intake, gm./rat/day	% Mortality after	
		500 turns	800 turns
dl-α-Alanine	0.200	83 (6)*	—
β-Alanine	0.169	100 (3)	—
l-Arginine	0.228	66 (3)	—
l-Cystine	0.050	66 (3)	—
l-Cystine	0.100	100 (3)	—
l-Glutamic acid	0.330	—	100 (3)
l-Glutamic acid	0.290	33 (6)	—
Glycine	0.306	66 (3)	—
l-Histidine	0.066	100 (3)	—
l-Histidine	0.130	33 (3)	—
l-Histidine	0.130	—	100 (3)
l-Histidine	0.133	0 (3)	—
l-Histidine	0.214	—	100 (3)
l-Histidine	0.293	—	100 (3)
dl-Isoleucine	0.200	66 (3)	—
l-Leucine	0.140	100 (3)	—
dl-Methionine	0.100	33 (3)	—
dl-Norleucine	0.066	—	100 (3)
dl-Norleucine	0.088	0 (3)	—
dl-Norleucine	0.100	—	100 (3)
dl-Serine	0.083	66 (3)	—
dl-Serine	0.133	33 (3)	—
dl-Threonine	0.100	100 (3)	—
l-Tyrosine	0.133	66 (3)	—
dl-Valine	0.174	—	100 (3)
dl-Valine	0.176	—	66 (3)
Arginine and histidine	0.636	—	100 (3)
Arginine, histidine, lysine	0.520	—	100 (3)
Isoleucine, leucine, methionine, phenylalanine, threonine	0.280	—	100 (3)
Alanine, arginine, aspartic, cystine, glycine, histidine, lysine, serine	0.642	—	100 (3)
Choline	0.133	50 (2)	
Choline	0.226	66 (3)	—
Creatine	0.400	50 (3)	—
Creatine and methionine	0.500	100 (3)	—

* *Number of rats given in parentheses.*

was used as replacement therapy. With a daily intake of 1 to 1.5 gm. per day an increase in weight of 1.6 to 3.0 gm. per day occurred. After protein depletion for 16 days this mixture fed for five days did not afford protection. However, with less severe depletion (seven days) and longer replacement (seven days) the mixture reduced the mortality to 33% compared with 86% for untreated animals.

Comparison of the Protective Effect of Gelatin and Equivalent Amounts of Nitrogen Administered as Amino Acids

It seemed possible in view of the preceding results that the protective action of gelatin, not being explicable by its content of any individual amino acid, might be related simply to the total amount of nitrogen administered. A set of experiments were designed to test this hypothesis and the results are shown in Table VII.

TABLE VII

EFFECT ON TOLERANCE TO TRAUMA OF FEEDING NITROGEN AS AMINO ACIDS FOR TWO WEEKS

Source of nitrogen in diet	Average nitrogen intake, gm./rat/day	% Mortality after 500 turns
None	—	72 (18)*
Gelatin	1.35	25 (12)
Glutamic acid and glycine	1.38	25 (12)
Glutamic	0.68	100 (4)
Glycine	0.29	66 (6)
Glycine	0.60	66 (6)

* *Number of rats given in parentheses.*

It may be seen that control animals for two weeks on the protein-free diet showed a mortality of 72% after trauma. In a group of animals fed 10% gelatin (Diet C-7) over a similar period the mortality was only 25%. A third group were fed a mixture of *l*-glutamic acid and glycine (the proportion being 93 and 7%, respectively). This diet contained 18% nitrogen incorporated in Diet C, the total nitrogen intake being the same as that of the gelatin group. In this case the mortality was again 25%. When smaller amounts of either glycine or *dl*-glutamic acid were given, little or no protective action was seen.

Discussion

The results described in this paper are a continuation of those in the preceding paper. Experiments have been outlined in which various protein diets were used in an attempt to reduce the increased susceptibility to trauma of protein-depleted rats. After a depletion period of two weeks or less a definite reduction in mortality occurred by the fifth day of protein feeding and in most cases was maximal for any given diet by the sixth day. This improvement was generally unrelated to the daily weight gain and maximal protection was usually observed before the animals regained their lost weight. Levels of

15% protein with an intake of between 1 and 1.5 gm. per day were as effective as 30% with twice the intake. The length of time of replacement rather than higher levels of protein appeared to be the important factor.

In certain cases, however, with amounts of protein between 0.08 gm. and 0.68 gm. per day, there was a definite relation between intake and tolerance to trauma. Mortality after 500 turns in the drum dropped sharply with increasing intake in this range but showed little further improvement after intakes of between 1 and 2 gm. When the trauma was increased to 800 turns, little protection was found, possibly because of the limited time over which the protein diets were fed. Casein, Amigen, and wheat germ protein when effective for replacement therapy, all appeared to be equally so at the same intake levels.

When the rats were fed a protein-free diet for more than a month, two weeks of protein feeding were required to effect a definite reduction in susceptibility to trauma. In some cases the animals had regained their normal weight and appeared in excellent condition, yet still showed a high mortality after trauma. Three weeks of protein feeding was required to restore tolerance to normal levels.

In some experiments a deficient diet containing gelatin allowed an increase in protection to take place after protein depletion. Some amino acids and the Vuj mixture in one experiment showed some protection against trauma of 500 turns. None afforded protection against more severe trauma. Unfortunately these experiments were done before the importance of longer replacement periods was appreciated. It is possible that longer feeding of amino acid diets might have given more clear-cut results. On the basis of the present experiments no one amino acid or even all the essential amino acids given at a high level have been demonstrated conclusively to afford as good protection as low levels of casein or gelatin that would contain comparatively much smaller amounts of any one of the amino acids except glycine.

In a small group of animals having an intake of amino acids (glutamic and glycine) equivalent in nitrogen to a 10% gelatin diet, mortality was reduced to the level of that of the gelatin-fed rats. These experiments were unlike the others in that the animals were not previously depleted of protein before being put on the experimental diet. It would suggest that the protective action of deficient proteins is in some way related to the total nitrogen content rather than to the effect of any individual amino acid.

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